OMTN, Volume *35*

Supplemental information

Selective targeting of chemically modified miR-34a to prostate cancer using a small molecule ligand and an endosomal escape agent

Ahmed M. Abdelaal, Ikjot S. Sohal, Shreyas G. Iyer, Kasireddy Sudarshan, Esteban A. Orellana, Kenan E. Ozcan, Andrea P. dos Santos, Philip S. Low, and Andrea L. Kasinski

Supplemental Materials and Methods

Materials

1,2-diaminoethane trityl resin, amino acids and coupling reagents were purchased from Chem-Impex International (Chicago, IL). Nigericin sodium salt and DBCO-NHS Ester were purchased from Cayman Chemical Company (Ann Arbor, MI), Broadpharm (San Diego, CA) respectively. H-Cys(Trt)-2-chlorotrityl resin was obtained from Novabiochem (San Diego, CA). Perchloric acid, TFA, MeOH, IPA, DMSO, DMF, DIPEA, piperidine, DCM, Et_2O, K_2CO_3, and all other chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO). All preparative HPLC was performed with an Agilent 1200 Instrument with a reverse-phase XBridge OBD preparative column (19 × 150 mm, 5 μm) manufactured by Waters (Milford, MA) with UV detection at 254 nm. LRMS LC/MS was performed on an Agilent 1220 Infinity LC with a reverse-phase XBridge Shield RP18 column (3.0 × 50 mm, 3.5 μm).

Synthesis of DUPA-Peptide-NH_2

DUPA-Peptide-NH2 compound was prepared as previously reported(1). In brief, 1,2-diaminoethane trityl resin (0.96 mequiv/g, 2 g, 1.92 mmol) was swollen in CH_2Cl_2 (10 mL) and DMF (10 mL) for 30 min each, while argon was bubbled through the mixture. After draining the solvents, and a solution of Fmoc-Phe-OH (2.5 equiv), PyBOP (2.5 equiv), HOBt (2.5 equiv), and DIPEA (5.0 equiv) in DMF (10 mL) was added to the resin. Argon was bubbled through the mixture for 12 h, and the solvent was then drained. The resin was washed with DMF (15 mL × 3, drained after each wash) and ^{i}PrOH (15 mL × 3, drained after each wash). A Kaiser test was performed to give a negative result, which indicated the coupling reaction was successful. The resin was then washed with 20% piperidine in DMF (10 mL × 3, in 15 min/wash, drained after each wash), DMF (15 mL × 3, drained after each wash), and ^{i}PrOH (15 mL × 3, drained after each wash). A second Kaiser test was performed to give a positive result, which indicated the cleavage of the Fmoc group was successful. The above sequence was repeated for the coupling of Fmoc-L-Phe-

OH (2.5 equiv), Fmoc-8-Aoc-OH (Fmoc-8-aminocaprylic acid) (2.5 equiv), and the protected DUPA precursor[1] (2.5 equiv). The final product was cleaved from the resin by washing with a TFA/H_2O/TIPS cocktail (92.5:2.5:2.5) (10 mL × 3, 60 min), during which argon was bubbled through the mixture. Resin washed twice with cleavage mixture. The filtrate was collected and concentrated. Addition of Et_2O caused precipitation of a solid. The mixture was centrifuged, and the precipitate was collected. The crude product was purified by preparative RP-HPLC [λ = 254 nm; solvent gradient, 0% B to 80% B in 30 min at 40 mL/min; A = 20 mM ammonium acetate buffer at pH = 7; B = MeCN]. R_T = 2.3 min, ($M+H^+$ = 798.0).

DUPA-DBCO conjugate synthesis

To a stirred solution of DUPA-peptide NH_2 (30 mg, 0.0376 mmol, 1 eq.) in DMSO, NHS-DBCO (17 mg, 0.0414 mmol, 1.1 eq.) and DIPEA (7.2 mg or 0.1 mL, 0.056 mmol, 1.5 eq.) was added dropwise. The reaction mixture continued to stir at room temperature. Progress of the reaction was monitored by LC-MS. After the complete conversion of DUPA-Peptide NH_2, the crude reaction mixture was purified by RP-HPLC [λ = 254 nm; solvent gradient, 0% B to 80% B in 30 min at 40 mL/min; A = 20 mM ammonium acetate buffer at pH = 7; B = MeCN]. Yield = 49%, R_T = 2.3 min, ($M+H^+$ = 1086.0).

DUPA-Peptide-SH Synthesis

1,2-diaminoethane trityl resin (0.96 mequiv/g, 2 g, 1.92 mmol) was swollen in CH_2Cl_2 (10 mL) and DMF (10 mL) for 30 min each, while argon was bubbled through the mixture. After draining the solvents, and a solution of Fmoc-*S*-trityl-*L*-cysteine (2.5 equiv), PyBOP (2.5 equiv), HOBt (2.5 equiv), and DIPEA (5.0 equiv) in DMF (10 mL) was added to the resin. Argon was bubbled through the mixture for 12 h, and the solvent was then drained. The resin was washed with DMF (15 mL × 3, drained after each wash) and ^{i}PrOH (15 mL × 3, drained after each wash). A Kaiser test was performed to give a negative result, which indicated the coupling reaction was successful. The

resin was then washed with 20% piperidine in DMF (10 mL × 3, in 15 min/wash, drained after each wash), DMF (15 mL × 3, drained after each wash), and ^{i}PrOH (15 mL × 3, drained after each wash). A second Kaiser test was performed to give a positive result, which indicated the cleavage of the Fmoc group was successful. The above sequence was repeated for the coupling of Fmoc-L-Phe-OH (2.5 equiv) (twice), Fmoc-8-Aoc-OH (Fmoc-8-aminocaprylic acid) (2.5 equiv), and the protected DUPA precursor[1] (2.5 equiv). The final product was cleaved from the resin by washing with a TFA/H_2O/TIPS cocktail (92.5:2.5:2.5) (10 mL × 3, 60 min), during which argon was bubbled through the mixture. Resin washed twice with cleavage mixture. The filtrate was collected and concentrated. Addition of Et_2O caused precipitation of a solid. The mixture was centrifuged, and the precipitate was collected. The crude product was purified by preparative RP-HPLC [λ = 254 nm; solvent gradient, 0% B to 80% B in 30 min at 40 mL/min; A = 20 mM ammonium acetate buffer at pH = 5; B = MeCN]. R_T = 2.5 min, ($M+H^+$ = 902.0)

Nigericin Pyridyl disulfide amide synthesis

Nigericin sodium salt (100 mg, 0.13386 mmol) was stirred with 1N $HClO_4$ (1 mL) for 1 h in $CHCl_3$ (1 mL), washed with water (2 × 25 mL), extracted with $CHCl_3$ (3 × 25 mL) and dried over anhydrous Mg_2SO_4. The organic extract was filtered and evaporated to provide nigericin free acid which was used without further purification. Nigericin free acid (0.14 mmol), Py-SS-$(CH_2)_2NH_2$ (0.21 mmol), HATU (0.21 mmol), and DIPEA (0.28 mmol) were dissolved in anhydrous CH_2Cl_2 (3.0 mL) and stirred under argon at room temperature for 12 h. Progress of the reaction was monitored by LCMS. After complete conversion of nigericin free acid, the crude reaction mixture was subjected to purification by RP-HPLC, (mobile phase A = 20 mM ammonium acetate, pH = 7; organic phase B = acetonitrile; method: 0% B to 100% B in 35 minutes at 40 mL/min) and furnished nigericin-SS-amide derivative 50% yield. LC-MS (A = 20 mM ammonium bicarbonate, pH = 7; organic phase B = acetonitrile; method: 0% B to 100% B in 15 minutes) R_T = 9.15 min ($M+NH4^+$ = 910.5).

DUPA-SS-Nigericin Synthesis

To a stirred solution of DUPA-Peptide-SH (36mg, 0.040310 mmol, 1.5 eq.) and Pyridyl disulfide amide derivative of Nigericin (24 mg, 0.02687 mmol, 1.0 eq.) in DMSO (1 mL), DIPEA (7 uL) was added dropwise. The reaction mixture continued for stirring at room temp. Progress of the reaction was monitored by LCMS. After the complete conversion of DUPA-Peptide NH_2, the crude reaction mixture was purified by RP-HPLC [λ = 254 nm; solvent gradient, 0% B to 80% B in 30 min at 40 mL/min; A = 20 mM ammonium acetate buffer at pH = 7; B = MeCN], Yield = 45%, R_T = 5.5 min, (M^+ = 1683.0).

DUPA-SS-DBCO-Nigericin Synthesis

To a stirred solution of DUPA-SS-Nigericin-NH_2 (7 mg, 0.004159 mmol, 1 eq.) and NHS-DBCO (2 mg, 0.0054070 mmol, 1.3 eq.) in DMSO (o.5 mL), DIPEA (1 uL, 1.5 eq.) was added dropwise. The reaction mixture continued for stirring at room temp. Progress of the reaction was monitored by LCMS. After the complete conversion of DUPA-Peptide NH_2, the crude reaction mixture was purified by RP-HPLC [λ = 254 nm; solvent gradient, 0% B to 80% B in 30 min at 40 mL/min; A = 20 mM ammonium acetate buffer at pH = 7; B = MeCN]. Yield = 51%, R_T = 5.8 min, ($M+H^+$ = 1970).

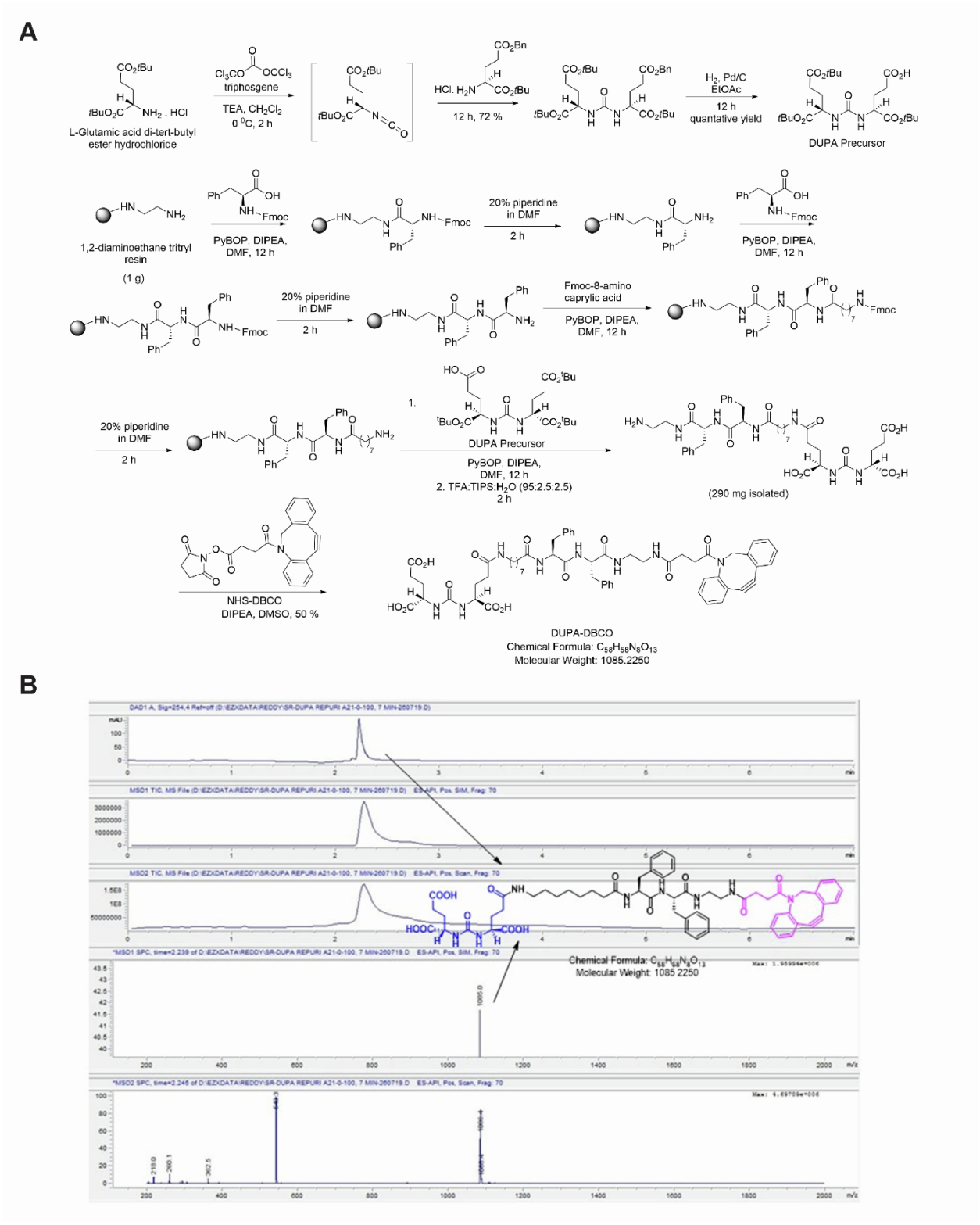

Figure S1. DUPA-dbco conjugate synthesis and validation. A) Synthesis of DUPA-dbco by solid phase peptide synthesis method. **B)** LC-MS spectrum of DUPA-DBCO conjugate.

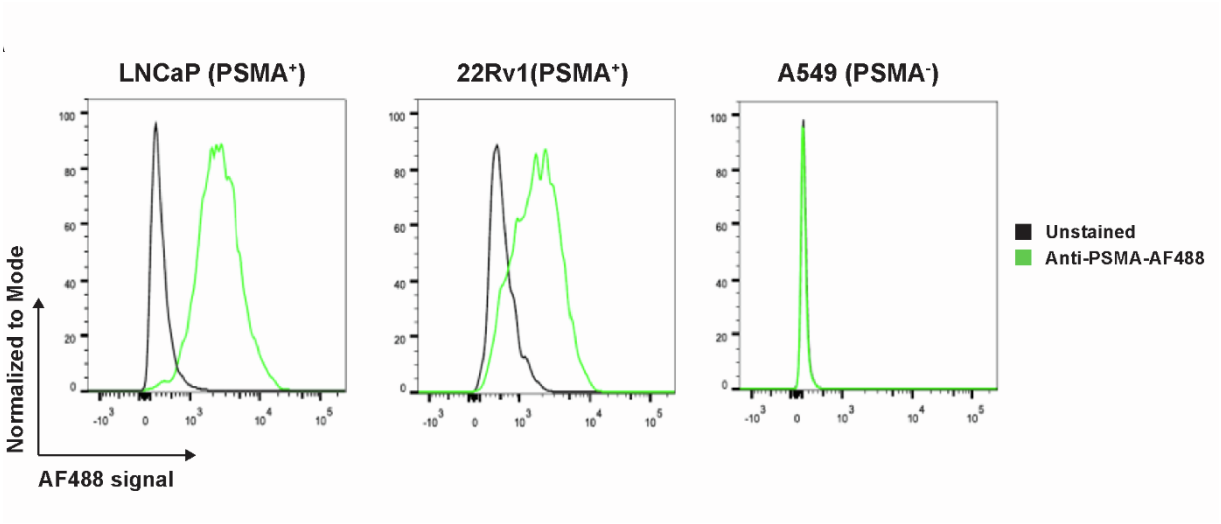

Figure S2. Validation of PSMA expression. Flow cytometry histograms indicate the expression of PSMA in LNCaP cells and 22Rv1 cells but not A549 cells.

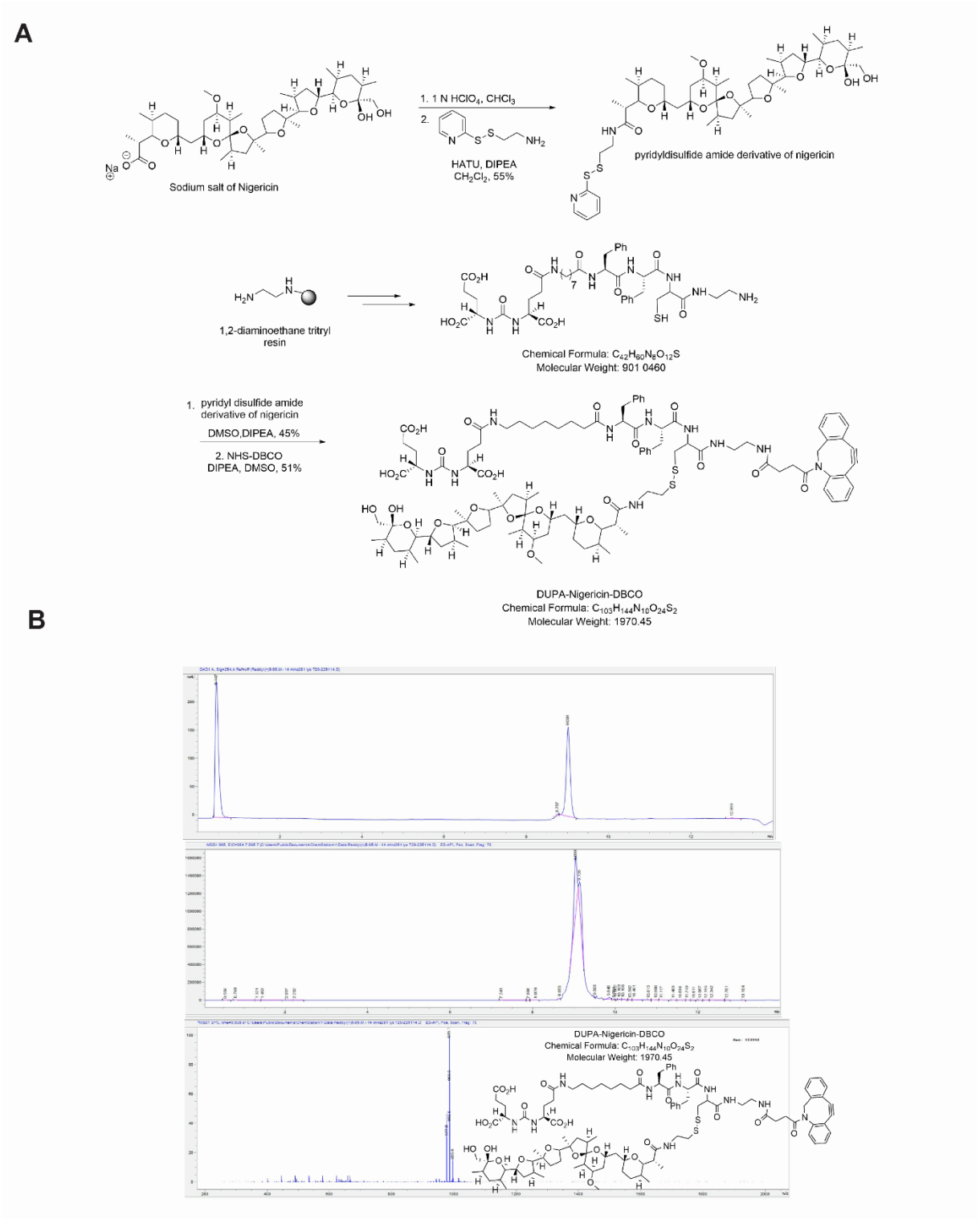

Figure S3. DUPA-nigericin-dbco conjugate synthesis and validation. A) Synthesis of DUPA-nigericin-dbco by solid phase peptide synthesis method. **B)** LC-MS spectrum of DUPA-nigericin-dbco conjugate.

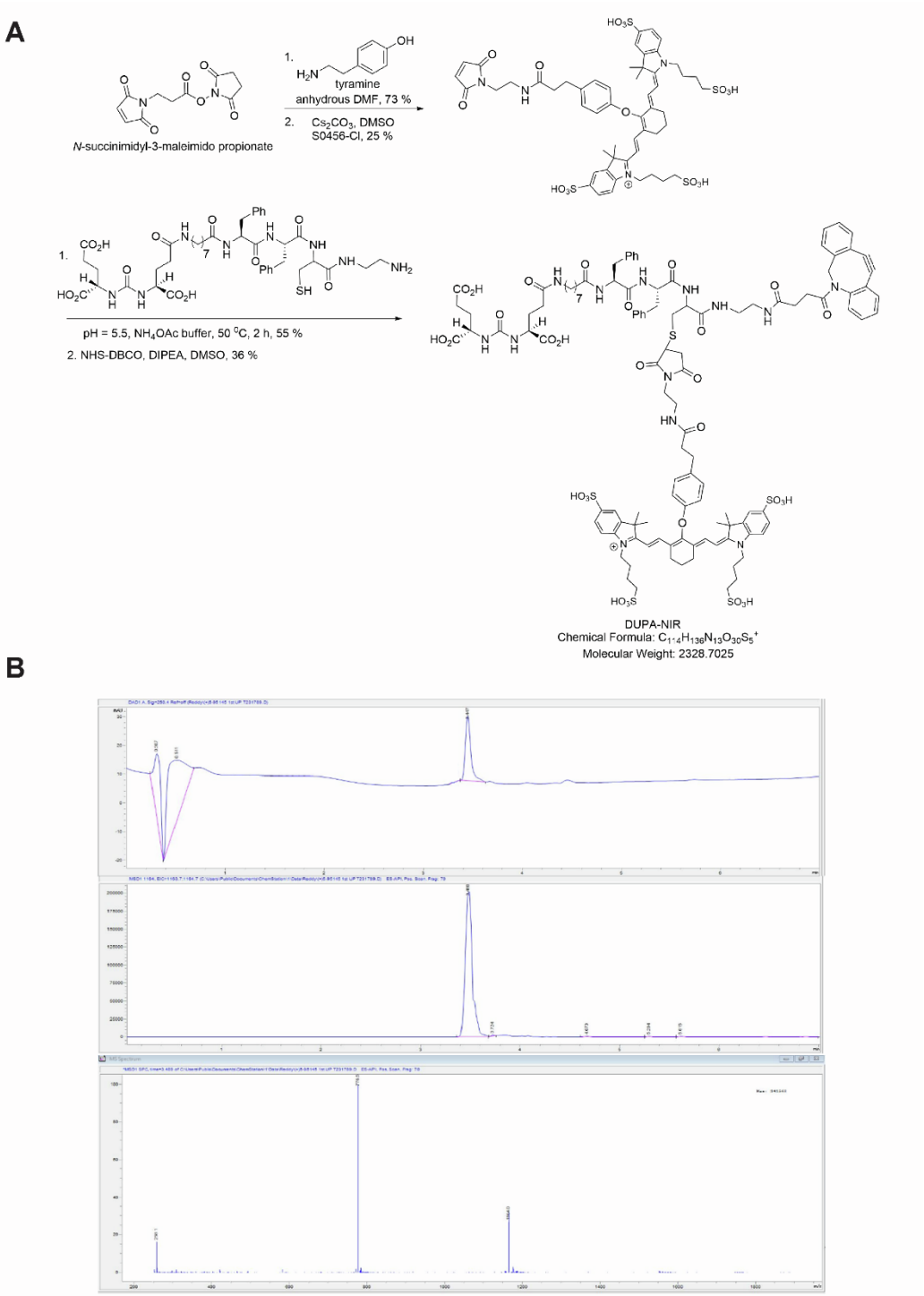

Figure S4. DUPA-NIR conjugate synthesis and validation. A) Synthesis of DUPA-NIR by solid phase peptide synthesis method. **B)** LC-MS spectrum of DUPA-NIR conjugate.

References:

1. Roy,J., Nguyen,T.X., Kanduluru,A.K., Venkatesh,C., Lv,W., Reddy,P.V.N., Low,P.S. and Cushman,M. (2015) DUPA Conjugation of a Cytotoxic Indenoisoquinoline Topoisomerase i Inhibitor for Selective Prostate Cancer Cell Targeting. *J Med Chem*, **58**, 3094–3103.